

Zero methane emission bogs: extreme rhizosphere oxygenation by cushion plants in Patagonia

Christian Fritz^{1,2}, Veronica A. Pancotto³, Josephus T. M. Elzenga², Eric J. W. Visser⁴, Ab P. Grootjans^{2,5}, Arjan Pol⁶, Rodolfo Iturraspe⁷, Jan G. M. Roelofs¹ and Alfons J. P. Smolders¹

¹Department of Environmental Biology, Institute for Water and Wetland Research (IWWR), Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, the Netherlands; ²Laboratory of Plant Physiology, University of Groningen, PO Box 14, 9750 AA, Haren, the Netherlands; ³Ecology Group, CADIC-CONICET, B. Housay 200 (9410) cc92, Ushuaia, Tierra del Fuego, Argentina; ⁴Department of Experimental Plant Ecology, Institute for Water and Wetland Research, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, the Netherlands; ⁵Centre for Energy and Environmental Studies, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, the Netherlands; ⁶Department of Microbiology, Institute for Water and Wetland Research, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, the Netherlands; ⁷Dirección de Recursos Hídricos – Gobierno de Tierra del Fuego, San Martín 1401 (9410), Ushuaia, Tierra del Fuego, Argentina

Summary

Author for correspondence:

Christian Fritz

Tel: +31 24 3652337

Email: f.c@gmx.li

Received: 20 August 2010

Accepted: 23 November 2010

New Phytologist (2011)

doi: 10.1111/j.1469-8137.2010.03604.x

Key words: cushion plant, methane, nutrient, Patagonia, rhizosphere oxygenation, root, *Sphagnum*, wetland.

- Vascular wetland plants may substantially increase methane emissions by producing root exudates and easily degradable litter, and by providing a low-resistance diffusion pathway via their aerenchyma. However, model studies have indicated that vascular plants can reduce methane emission when soil oxygen demand is exceeded by oxygen released from roots. Here, we tested whether these conditions occur in bogs dominated by cushion plants.
- Root–methane interactions were studied by comparing methane emissions, stock and oxygen availability in depth profiles below lawns of either cushion plants or *Sphagnum* mosses in Patagonia.
- Cushion plants, *Astelia pumila* and *Donatia fascicularis*, formed extensive root systems up to 120 cm in depth. The cold soil (< 10°C) and highly decomposed peat resulted in low microbial activity and oxygen consumption. In cushion plant lawns, high soil oxygen coincided with high root densities, but methane emissions were absent. In *Sphagnum* lawns, methane emissions were substantial. High methane concentrations were only found in soils without cushion plant roots.
- This first methane study in Patagonian bog vegetation reveals lower emissions than expected. We conclude that cushion plants are capable of reducing methane emission on an ecosystem scale by thorough soil and methane oxidation.

Introduction

Effects of vascular plants on methane cycling in wet soils

Wetlands are favourable habitats for methanogenic archaea that form methane during the decomposition of organic material. These methanogens require environments with no oxygen and abundant organic matter, both of which are present in wetland conditions (Segers, 1998). Peatlands dominated by *Sphagnum* mosses (bogs) are known for the slow decomposition of dead organic matter. The refractory nature of *Sphagnum* litter is mainly responsible for this slow

decomposition, as other plants, including typical bog species, decompose much more rapidly than mosses (Aerts *et al.*, 1999; Woodin *et al.*, 2009). Therefore, if nutrient availability permits the dominance of vascular plants, the potential production of methane is strongly increased by a high production of vascular plant biomass, which results in an increased input of more easily decomposable litter (Whiting & Chanton, 1993; Joabsson & Christensen, 2001). Underground vascular plant tissue can also transport labile carbon compounds into anoxic soil layers (Joabsson & Christensen, 2001; Ström *et al.*, 2003; Chanton *et al.*, 2008). Such increased substrate stocks for methanogenic archaea may be crucial because methane production is

frequently substrate limited (reviewed in Whalen, 2005). Moreover, aerenchymatous roots can strongly stimulate the export of methane by creating shortcuts to the atmosphere (van der Nat & Middelburg, 1998; Kutzbach *et al.*, 2004; Whalen, 2005).

The presence of roots, however, may also decrease the release of methane. Oxygen diffuses through the aerenchyma of vascular plants from the atmosphere into the roots and subsequently leaks into the rhizosphere (Armstrong *et al.*, 1991, 2006). Under such oxic rhizosphere conditions, methane production can be reduced by two orders of magnitude (reviewed in Segers, 1998). In addition, when oxygen is present, methane stocks can be decreased by oxidation via methanotrophic bacteria (King, 1994; Sorrell *et al.*, 2002; Raghoebarsing *et al.*, 2005). The passage through a thick aerobic soil–atmosphere interface (i.e. 3–20 cm of aerobic soil) can thus oxidize most of the methane (Roulet *et al.*, 1993; Daulat & Clymo, 1998; Hornibrook *et al.*, 2009). Our study investigated methane release from bog lands that have high root densities, and provides evidence that certain wetland ecosystems do not produce nearly as much methane as do most temperate and tropical wetlands.

The extent to which the rhizosphere can become aerated depends on various conditions: root density, rate of oxygen loss from the roots, soil oxygen consumption and the diffusion coefficient of oxygen in the soil. Under most conditions, the combination of high oxygen consumption (high temperature, suitable substrate and high microbial activity) and limited oxygen release (limited oxygen conduction capacity and low root density) will result in a very thin oxic rhizosphere. Therefore, in wetland soils, a large fraction of the substrate surrounding a root remains anoxic despite root oxygen loss (Armstrong *et al.*, 1991, 1992). Such incomplete oxidation of organic soils promotes the coexistence of roots and methane (Grosse *et al.*, 1996), ultimately resulting in increased emission of methane (Watson *et al.*, 1997; Ding *et al.*, 2005).

Depending on the type of vegetation, the potential to lower methane emission by the creation of oxic soil conditions varies from 16% to 95% (Laanbroek, 2009). Extensive rhizospheric oxidation requires a dense root biomass (Grosse *et al.*, 1996; Smolders *et al.*, 2002), which, in turn, provides extra carbon for methane production. However, many studies ignore such additional methane production fuelled by plant litter and root exudates when estimating the oxidation potential of the rhizosphere. The root–methane interaction model of Watson *et al.* (1997) required high root biomass to find considerable methane oxidation. Only thorough rhizospheric oxidation created a sufficiently large spatial separation of roots from methane to prevent aerenchyma-mediated diffusion (Grosse *et al.*, 1996). Methane emissions can become temporarily decoupled from vascular plant cover when the water levels are low (Bubier, 1995; Couwenberg *et al.*, 2010). At these dry sites,

the water table drops below the bulk root mass, so that methane is oxidized before being released via plants.

In essence, the large majority of studies show that vascular plants increase methane release from wetlands (Whiting & Chanton, 1993; Waddington *et al.*, 1996; Kutzbach *et al.*, 2004; Bortoluzzi *et al.*, 2006). Estimations reveal that approximately one-third of global methane emission derives from wetlands, where minerotrophic wet peatlands and marshes dominated by vascular plant vegetation are the most important sources (Whiting & Chanton, 1993; Saarnio *et al.*, 2009; Koelbener *et al.*, 2010). Bypassing the aerobic soil–atmosphere interface will be the main cause for high, vascular plant-mediated emission rates. Via their aerenchyma, higher plants can conduct 50–95% of the total methane emission (Ding *et al.*, 2005; Whalen, 2005).

Global climate change leading, for instance, to nutrient availability and changes in soil wetness is believed to increase the vascular plant cover in peatlands (Johansson *et al.*, 2006; Breeuwer *et al.*, 2010), which substantially feeds back to methane cycling in wetlands (see the first two paragraphs of the Introduction). However, the importance of particular plant species in methane cycling remains highly variable (Joabsson *et al.*, 1999; Laanbroek, 2009). Part of this variation can be explained by the varying dominance of plant functional types as shown in recent studies (Bouchard *et al.*, 2007; Kao-Kniffin *et al.*, 2010; Koelbener *et al.*, 2010). Plant functional types (reviewed in Ustin & Gamon, 2010) may efficiently combine differences in traits, such as litter production, root density and oxygenation potential (Sorrell *et al.*, 2001; Allen *et al.*, 2002; van Bodegom *et al.*, 2005; Bouchard *et al.*, 2007). The functional type ‘cushion plant’ (cf. Gibson & Kirkpatrick, 1985) has not been studied with respect to methane, despite its importance in forming peatlands in the Southern Hemisphere.

We hypothesized that in, vascular plant-dominated wetlands, methane release may be decreased or even absent if the soil is thoroughly oxidized by extensive rhizosphere oxygen loss. In search of such wetlands, we targeted methane-producing wetlands (e.g. deep bogs) where oxygen consumption is low. The rainy cold parts of Patagonia harbour pristine bogs with very few nutrients (Kleinebecker *et al.*, 2008; Schmidt *et al.*, 2010). Darwin (1839) described bogs in Patagonia with deep rooting plants growing as dense cushion-like vegetation that formed extensive blanket bogs. Cushion plants, such as *Astelia* ssp. and *Donatia* ssp., form dense root systems consisting of shallow tap roots and aerenchymatous roots of > 100 cm in length (Darwin, 1839; Grootjans *et al.*, 2010). Our objective was to elucidate whether these roots negatively affected methane emission by comparing densely rooted sites with sites covered only by moss species (*Sphagnum* ssp.). Interactions of roots with soil methane cycling were studied by correlating the vertical distribution of methane stock, oxygen availability and methane oxidation potential with the root biomass density of cushion plants.

Materials and Methods

Sampling design and description of experimental sites

The effects of roots on methane (CH_4) were studied by comparing methane dynamics in different bog vegetation in southernmost Patagonia: cushion plant lawns in a cushion bog (high root biomass); *Sphagnum magellanicum* lawns adjacent to the cushion plant lawns mentioned above (non-rooted sites in a system dominated by roots); *Sphagnum magellanicum* lawns in a control bog with a cover of vascular plants less than 1% (nonrooted sites in a system with only a few roots). Each type was represented by three replicates. In addition, we included two pools surrounded by cushion plants, but without roots in the soil, to estimate methane emissions independent from atmospheric oxygen (oxidation) and roots (gas transport).

Field measurements and experiments were performed in a cushion bog peatland in Tierra del Fuego (Moat, 54°58'S; 66°44'W, 40 m asl) where average daily air temperatures are 5–6°C with cold summers (maximum average temperature, 9°C; R. Iturraspe & C. Fritz, unpublished). July is usually the coldest month at 2°C. The absence of a thermal summer is typical for oceanic bogs in Patagonia (Kleinebecker *et al.*, 2007 and literature therein). The control bog (Andorra, 54°45'S; 68°20'W, 200 m asl) shows slightly higher daily and seasonal temperature differences during the summer because of its location at a valley bottom (Iturraspe *et al.*, 1989). The soil temperature was low and stable at both bogs throughout the growing season, decreasing from 8 to 12°C at 5 cm below the surface to 4–8°C at 100 cm depth. Soil temperature profiles were recorded during expeditions in spring 2006 and summer 2007. Annual precipitation assessed in the 1980s and from 2008 onwards exceeded 60 cm, evenly distributed over the year in both peatlands, providing wet conditions (Iturraspe *et al.*, 1989; R. Iturraspe & C. Fritz, unpublished). Water levels fluctuated between 5 cm above and 20 cm below the surface at all lawn sites from spring to autumn.

The cushion bog was dominated by lawns of evergreen cushion plants intermingled with patches (few square metres) of dominating *Sphagnum magellanicum* (Bridel) and scarcely vegetated pools (Roig & Collado, 2004; Gebser, 2008). Dominating cushion plants were *Astelia pumila* (G. Forster) R. Br. and *Donatia fasciculata* R. Br. et G. Forster covering > 70%. The soil below cushion plants was densely packed with tap roots (1–2 mm diameter) and fine roots exceeding depths of 120 cm (Grootjans *et al.*, 2010). By contrast, roots and vascular plants were almost absent at *Sphagnum* sites. The peat depth was comparable between sites, ranging from 700 to 1000 cm, thus providing large stocks of carbon-rich substrate. The densely rooted cushion plant peat was highly decomposed (H8–H10 on the Von-Post scale), contrasting with the well-preserved *Sphagnum* peat (Kleinebecker *et al.*,

2007; Gebser, 2008). Peat formed by cushion plants was three to five times denser than *Sphagnum* peat. At all sites, *Sphagnum* peat was found at depths greater than 300 cm. The peatlands studied remained unaffected by anthropogenic alteration, such as drainage, agricultural use or elevated atmospheric nutrient deposition. Reviewing scarce deposition data from Patagonia, Godoy *et al.* (2003) suggested bulk nitrogen depositions below 0.1 g N m⁻² a⁻¹ in coastal regions. The substrate was very low in nutrients, with total phosphorus concentrations typically below 0.023% in *Sphagnum* peat and 0.034% in cushion plant peat. Pore water reflected acidic conditions in both bogs (pH 3.8–4.2) with little variation in the upper 300 cm.

Methane/ethane concentration measurements

Methane and ethane headspace samples were measured on a Hewlett-Packard® (Avondale, California, USA) 5890 gas chromatograph equipped with a flame-ionization detector and a Porapak Q column (80/100 mesh), operated at 120°C with nitrogen as carrier gas, in the laboratory of Radboud University, Nijmegen, the Netherlands (accuracy, 0.2 ppm). The injection volume was 0.1 ml for incubations and pore water samples and 0.5 ml for emission samples to improve the detection of low concentrations.

Methane dynamics (stock and emission)

Methane stock and release were estimated by means of pore water concentration and emission into static chambers, respectively. Sampling took place over the growing season: December 2008 (spring), February 2009 (summer) and late March 2009 (autumn). For logistic reasons, sampling was delayed for 1 wk in the control bog. Insights into inter-annual and seasonal variations in methane stock were addressed by sampling pore water eight times from 2006 to 2009 at one site per vegetation type.

Pore water samples were drawn from eight depths (5, 30, 60, 120, 150, 180, 300 and 600 cm) in the cushion bog and from five depths (5, 50, 150, 300 and 500 cm) in the control *Sphagnum* bog. Anaerobic peat water samples were taken using 5-cm ceramic cups (Eijkelpoort Agrisearch Equipment®, Giesbeek, the Netherlands), connected to vacuum infusion flasks (40 ml) after sampling 150 ml to exclude internal stagnant sampler water. The 40-ml glass infusion flasks had a sample to headspace ratio of, usually, 1 : 2. As internal standard, 1 ml of ultrapure ethane gas (Airliquide®, Eindhoven, the Netherlands) was added after sampling and flasks were stored at 4°C during < 2 wk until analysis. Microbial modification of samples was hampered by the addition of 0.1 mg HgCl₂ (0.1 ml of 0.1 g l⁻¹). Methane and ethane concentrations were measured in the headspace after vigorous shaking, releasing > 96% of methane to the headspace.

Methane emissions were assessed using dark static polyvinyl chloride (PVC) chambers (3700 cm³, 15 cm high) with bleeds of 4-mm PVC hose. PVC frames were installed 2 months before measurement and removable chamber tops were sealed to the frame by the water-filled rim. Gas samples were taken in the morning and in the afternoon on the same day at all sites per peatland. After placing the chambers, the temperature differed by < 3 K between $t = 0$ and the end of sampling. Gas samples were taken with a double-sided needle for 60 min in 20-min intervals in a pre-vacuumed 12-ml glass vial with a butyl stopper (Exetainer®, High Wycombe, UK). At sites with very low emissions, an additional sample was taken after 360 min. Samples were stored cool and analysed within 1 wk. Emission data are presented for 53 of 62 measurements where the linear slope fitted $r^2 > 0.75$ or when the methane headspace concentration stayed constant (zero emissions). Rejected time series, mostly pool sites, were probably subject to ebullition, as observed by unexpectedly high methane concentration at $t = 20$ min followed by depletion afterwards. After 2 months of additional storage, > 95% of original methane was retrieved in pore water bottles. Emission samples maintained equal concentrations. Methane release by large-scale ebullition was estimated by surface elevation fluctuations measured with water level recorders attached to the surface and a stable benchmark, as described by Fritz *et al.* (2008). Automatic recorders (Odyssey capacitance probes®, Dataflow, Christchurch, New Zealand) were set up to measure levels in 1-h intervals during 2 yr and confirmed with hand measurements during field visits.

Redox potential and oxygen measurements

Redox potential measurements were taken at five depths (30, 60, 120, 150 and 200 cm), 2–3 d after gas sampling. Per depth, four platinum electrodes were gently pushed into a pre-made hole and allowed to equilibrate. Stable readings were generally obtained after 30–60 min. In most cases, the drift was smaller than 1 mV min⁻¹ within 10 min. The redox potential (E_7) corrects the field measurements (E_{field}) for pH (pH_{soil}), absolute temperature in K (T) and the potential of the 3 M AgCl/Cl reference electrode ($E_{\text{ref}} = 217$ mV at 10°C) using the following relationship:

$$E_7 = E_{\text{field}} + E_{\text{ref}} + 0.2T(\text{pH}_{\text{soil}} - 7). \quad \text{Eqn 1}$$

Literature on redox processes (e.g. Laanbroek, 1990) suggested E_7 values of > 330–350 mV as an indication for free oxygen in soils. In figures showing redox data, we highlight 350 mV as a threshold for occurrence of free oxygen, also used by similar studies (Visser *et al.*, 2000). However, other studies found some nanomoles of oxygen for E_7 just above 300 mV (Lloyd *et al.*, 1998). Oxygen content in the soil was measured polarographically at 30 and 70 cm below the

water table in the cushion bog in February 2009. For oxygen measurements, we deployed platinum needle electrodes with a sensing tip of < 0.1 mm embedded in stainless steel (Microscale Measurements, The Hague, the Netherlands). The platinum tips remained protected by cellulose-nitrate membranes. Oxygen electrodes were connected to a custom-made nA-meter (Electronic Workshop, University of Groningen, the Netherlands) and an AgCl/Cl reference electrode. To calibrate, we used oxygen-saturated bog water in the field. Zero point calibration was performed in laboratory demineralized water flushed with nitrogen for at least 24 h. Persistent precipitation prevented frequent measurement of oxygen and also the establishment of polarograms at various depths. We measured currents at some 450-mV pre-settings of the equipment obtained from polarograms in Dutch bogs.

Root characteristics

Root density at the cushion plant site was determined by sampling in a piston corer [internal diameter (ID), 10 cm] to a depth of 80 cm and from 50 to 250 cm using a D-Section corer (ID, 4.7 cm; Eijkelkamp Agrisearch Equipment®, Giesbeek, the Netherlands). Roots were dried at 70°C for 2 d. Root density is expressed in gram per litre of soil. The presence of living fine roots of cushion plants (1–2 mm in diameter) usually coincided with a sharp change from black coloured peat to yellow–brown peat below the (oxygenated) rooting zone. In this article, the rooting zone comprises the entire volume of soil down to the maximum root depth. The proportion of rhizosphere to rooting zone depends on the root density and the space that is affected by the activity of individual roots. Integration of the root density over the entire rooting zone rendered the total dry root biomass expressed as g m⁻². The porosity of root material was determined in 1-cm increments using the microbalance method (Visser & Bögemann, 2003). To visualize oxygen loss in the rooting zone, we exposed cushion plants to an anaerobic methylene blue solution (25 mg l⁻¹ methylene blue, 0.5 g l⁻¹ agar, 5 mM KCl, 0.05 mM CaSO₄) filled in glass cuvettes in the laboratory. Sodium dithionite (Na₂S₂O₄) was used to decolorize the dye. The leaves projected into the air, but the surface of the solution was protected from the air by plastic and gently flushed with nitrogen (adapted after Armstrong *et al.*, 1992).

Clipping experiment

To highlight the functional role of cushion plants with respect to the redox state of the soil, the oxygen transport below the water table was hampered by removing the green parts of cushion plants in January 2008. We chose to remove four large areas of 2 × 2 m to reduce the effects of surrounding cushion plants. Differences in methane dynamics were

documented by methane pore water samples taken at three depths (60, 150 and 300 cm) after 1 month, 13 months and 26 months, as described above. The redox potential (E_7) was measured before and 13 months after the removal of green parts. Methane emissions were measured at three clipped sites 2 yr after clipping. Regrowth was minimal within 2 yr, which highlights the harsh growing conditions.

Methane production and consumption

To estimate differences in potential methane production and consumption, we took peat cores in the cushion bog (6 December 2008). Samples (100 ml) were placed in airtight plastic bags in the field and stored at 4°C before being processed in the laboratory. Differences in potential production were measured in one pure *Sphagnum magellanicum* and one cushion plant site dominated by *A. pumila* at 20, 70 and 120 cm depth. The activity of methanotrophs was estimated by incubating peat along a profile at cushion plant site 1. Samples were collected at depths of 70, 120, 140 and 150 cm. The maximum rooting depth was 140 cm at this site.

The interior of the bulk peat was subsampled by taking 20 g fresh weight of soil [some 1.2 g dry weight (DW)], which was incubated in 100-ml grey rubber-stoppered glass flasks threefold at 22°C. For potential methane production, flasks were flushed with nitrogen and vacuumed eight times to remove methane and oxygen. For aerobic production, we incubated with ambient air. Methane consumption incubations contained a headspace of ambient air and methane was added to a final concentration of 1.2–1.5%. Methane headspace concentration was frequently determined over 7 wk in both production and consumption incubation. Rates of methane production/consumption were derived from the linear part of the slope related to the weight of the sample after drying at 70°C for 48 h. For the cushion plant site, we present methane production rates for the beginning and end of the incubation period because rates differed by one order of magnitude. Oxygen depletion was regularly controlled by means of CO₂ headspace concentrations determined on an infrared gas analyser (IRGA, ABB Advance Optima, Zürich, Switzerland). Bulk density samples were taken at the same locations using the D-Section corer (ID, 4.7 cm) mentioned above, and dried at 70°C for 48 h. Rates of methane consumption and production are related to volume and to DW, respectively. Rates can be related to either the surface (volume) or substrate quality (DW).

Results

Methane emission and physical factors (temperature, water table)

Methane (CH₄) emissions were low, but significant, reflecting the low temperature and nutrient status of the bog sites

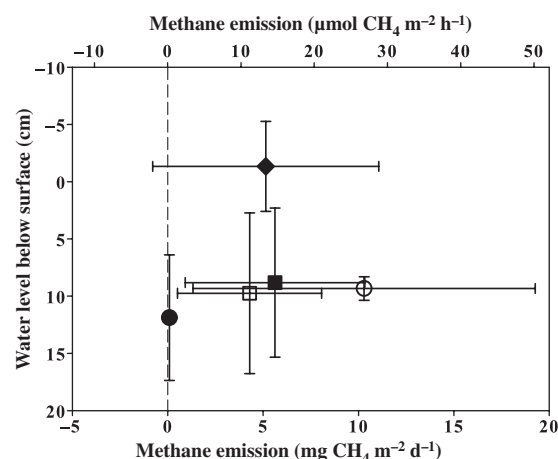


Fig. 1 Dependence of methane (CH₄) emission from various Patagonian bog vegetation types on water level in the soil. Emissions were not related to the water level, but varied with vegetation type and clipping treatment after 26 months (error bars indicate SD, $n = 6–16$). Cushion plant vegetation (closed circle) revealed zero emission when intact (dashed line), but highest emissions when clipped (open circle) ceasing oxygen transfer to the soil. *Sphagnum* lawns (closed square) had similar emission rates in the cushion bog as the control *Sphagnum* bog (open square) and pools (closed diamond). Emission measurements were taken in the morning and early afternoon using dark chambers in spring, summer and autumn, that is, December 2008, February 2009 and March 2009, respectively.

investigated (Fig. 1). In cushion plant lawns, however, emissions approached zero. One cushion plant site exhibited emission rates below 1 mg CH₄ m⁻² d⁻¹ on two occasions. In contrast with these virtually zero emissions, in *Sphagnum* lawns of the cushion bog the methane emission rate was 1–14 mg CH₄ m⁻² d⁻¹ (95% confidence interval), and similar to the control bog (1–11 mg CH₄ m⁻² d⁻¹). Emissions of pools were in the same range as those of *Sphagnum* lawns (Fig. 1). The highest emissions were found where cushion plants had been clipped. The average water levels during the measurements were comparable between the different sites (*c.* 5 cm, with cushion plant lawns being slightly drier), but fluctuated seasonally by some 10 cm from the mean water level (9 cm below the surface). Therefore, differences in water level did not correlate with methane emission rates ($r^2 = 0.05$, $n = 53$). The temperature was 8–12°C in air and 10°C in the first 10 cm of the soil, varying by < 2°C between measurements on the same day. Monitoring of surface elevation in cushion plant and *Sphagnum* lawns gave no indication of lifting of the peat surface by several centimetres within hours, which is associated with large-scale ebullition. Weak ebullition events could only be triggered in pools and *Sphagnum* growing in pools by jumping of the observer in the direct vicinity. The low frequency of ebullition generally indicates a low concentration of methane in the upper peat layers.

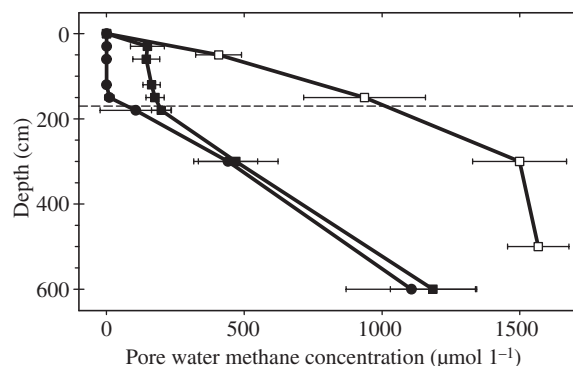


Fig. 2 Depth profile of methane (CH_4) stock concentrations in various Patagonian bog vegetation types. The rooting depth of cushion plants in the cushion bog is indicated by the dashed line. Methane was thoroughly depleted in the rooting zone below cushion plants (closed circles) and significantly lower in *Sphagnum* lawns in the cushion bog (closed squares) compared with the control *Sphagnum* bog (open squares). Error bars indicate SD, $n = 9$. The same sites and sampling intervals were used as in Fig. 1. Seasonal variation of methane stock was low.

Methane stock (pore water profiles)

Similar to emission rates, the methane stock varied strongly between different vegetation types, reflecting the presence of cushion plants and their deep roots (Fig. 2). Importantly, no methane ($< 1 \mu\text{mol l}^{-1}$) was found in the rooting zone of cushion plants, whereas *Sphagnum* lawns stocked less methane in the cushion bog compared with the control bog. The linear increase in methane concentration with depth was similar between the cushion bog and control bog; however, there was a 170-cm offset between the two bog types, which coincided with the maximum depth of the rooting zone in the cushion bog (Fig. 2). Unexpectedly, an offset in methane stock at this depth was also found in *Sphagnum* lawns < 3 m adjacent to cushion plants. In the upper 170 cm, the mean methane pore water concentration of $166 \mu\text{mol l}^{-1}$ ($\text{SD} = 46$, $n = 45$) remained stable with depth. This plateau of intermediate methane concentrations differed from the generally increasing methane concentrations with depth (Fig. 2). It needs to be stressed that *Sphagnum* patches formed small islands closely surrounded (< 3 m) by cushion plants and their rooting zone. Lateral gradients of methane pore water decreased over the same order of magnitude (50 – $150 \mu\text{mol CH}_4 \text{ l}^{-1} \text{ m}^{-1}$) as the gradients in depth (220 – $320 \mu\text{mol CH}_4 \text{ l}^{-1} \text{ m}^{-1}$). A levelled surface of 1% and hydraulic head differences of $< 0.2\%$ vertical (Gebser, 2008) suggested a substantial horizontal water movement. The horizontal methane gradients and water flow underline the connectivity of *Sphagnum* patches with their surrounding rooting zone of cushion plants. In addition, the intrusion of methane-depleted, oxic rain water most probably occurs in the sponge-like upper peat of *Sphagnum* lawns.

The absence of methane from the rooting zone of cushion plants becomes more visible when zooming at its lower

boundary. At all three cushion plant sites, the presence of methane was tightly linked to the lower boundary of the rooting zone of cushion plants. Remarkably, methane was always found only 5–10 cm below the maximum root depth, which differed between sites (Fig. 3a,b). Below 300 cm, many pore water samples indicated supersaturation of methane (mean, $1499 \mu\text{mol l}^{-1}$) in the control bog. Methane stock measurements taken between 2006 and 2009 revealed the same patterns. In general, seasonal and inter-annual variations were minor compared with the striking differences between the rooting zone of cushion plants and samples from nonrooted layers.

Presence of oxygen and roots

All three cushion plant sites were characterized by a dense root biomass (Fig. 3a,b). The average root biomass density was 2.15 g DW l^{-1} ($\text{SD} = 0.33$, $n = 3$) in the upper 170 cm. Integration of the root density along the rooting zone revealed that cushion plants maintained a total root biomass of 3590 g DW m^{-2} ($\text{SD} = 550$, $n = 3$). The porosity of roots of the dominating cushion plant *A. pumila* was 60–70%, providing sufficient aerenchyma for rapid diffusion of oxygen. Lower porosity was only found within 5 cm from the root tip (apex). Staining experiments with methylene blue suggested modest oxygen release rates along the length of the root, being highest around the root tips. Root tips could be found scattered over the entire depth profile. However, the largest densities of root tips were confined to the upper 70 cm, resulting in the highest potential to release oxygen in the upper half of the rooting zone (Fig. 3c). Less than 1% of the fine root biomass was located close (< 15 cm) to accumulated methane in the soil (Fig. 3b). At *Sphagnum* sites, the very few roots growing down to 30 cm reflected well the very sparse cover of vascular plants.

The decrease in redox potential mirrored the increase in methane, being highly sensitive to the presence of roots of cushion plants (Fig. 4). Free oxygen in the rooting zone to a depth of 120 cm was indicated by redox potentials higher than $E_7 = 330$ – 350 mV (cf. Laanbroek, 1990). The presence/activity of roots resulted in an increase in the redox potential of $c. 170$ mV compared with the *Sphagnum* site. Beyond the maximum rooting depth, the redox potential decreased rapidly to values comparable with *Sphagnum* sites. Root densities decreased strongly with depth, whereas redox potentials varied little in the upper 120 cm. By contrast, a tight relationship between root density and redox potential was found at the bottom of the rooting zone. At 150 cm depth, redox potentials varied substantially, $c. 364$ mV ($\text{SD} = 61$, $n = 9$), suggesting that a smaller proportion of the substrate remained aerated. Here, the coexistence of oxic and anoxic patches correlated with small numbers of root tips and low root densities (Fig. 3b). In the lower rooting zone, only two to five root tips were found per litre of peat

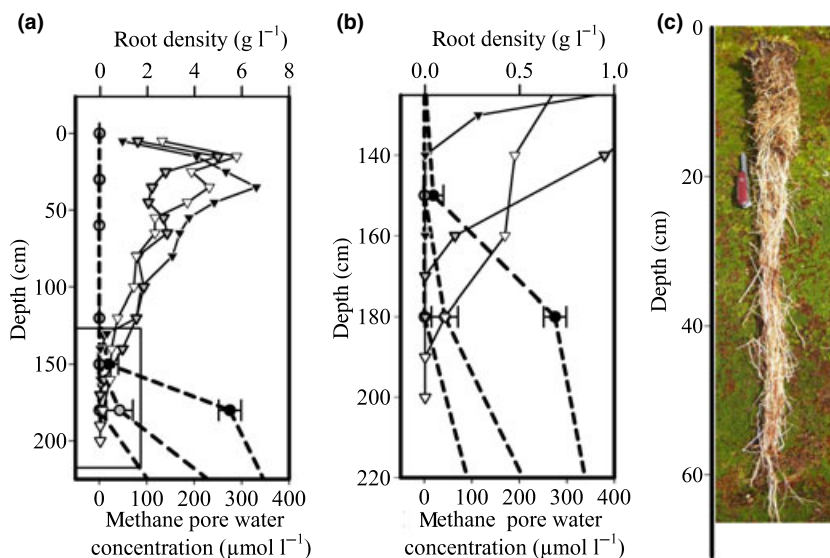


Fig. 3 Methane (CH₄) stocks (dashed lines) were inversely related to root density profiles (solid lines) in three individual cushion plant sites: CP1 (black symbols, 140-cm-deep roots), CP2 (grey symbols, 170-cm-deep roots) and CP3 (white symbols, 190-cm-deep roots). Methane was always found only 5–10 cm below the maximum root depth of individual sites. The box in (a) is, by approximation, the area of the graph expanded in (b). Same methane data are presented in Fig. 2. (c) Root biomass of cushion plants retrieved from the upper 70 cm using a piston corer (internal diameter, 10 cm).

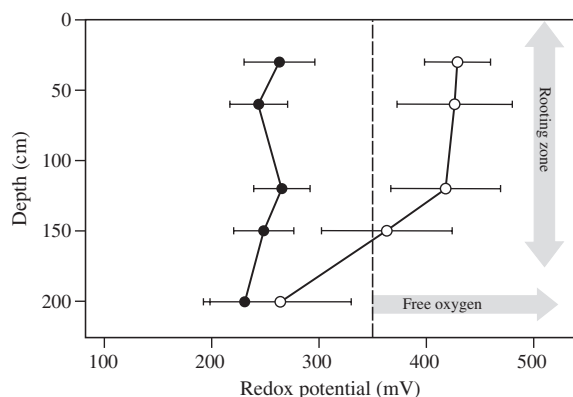


Fig. 4 Surplus oxygen in the rooting zone of cushion plants (open circles, $n = 9$) as indicated by a redox potential of > 350 mV (vertical dashed line). In *Sphagnum* lawns in the cushion bog (closed circles), the redox potential was $c. 170$ mV lower, suggesting anoxia. At each depth, the potential was measured by four electrodes. The same sites and sampling intervals were used as in Fig. 1. The vertical dashed line (350 mV) depicts the lower limit of the redox potential for oxygen-containing substrates (cf. Laanbroek, 1990).

substrate. Hence, a surplus of oxygen (leading to the presence of free oxygen) became more variable at these depths, leading to a high spatial variation in redox potential. Seasonal variations of the redox potential were small compared with differences related to the density/presence of roots. In February 2009, oxygen-sensitive mini-electrodes gave further evidence of oxygenated conditions. Oxygen concentrations up to $5 \mu\text{mol l}^{-1}$ were found at 30 cm and 70 cm below the water level in cushion plant lawns. Below

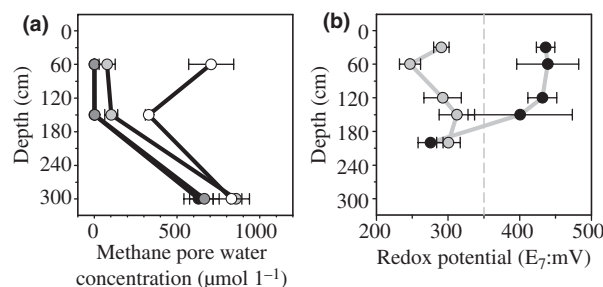


Fig. 5 Methane (CH₄) concentrations (a) increased in the soil after cushion plant plots ($2 \times 2 \text{ m}^2$) were clipped, resulting in soil anoxia (b). Methane accumulated over time ($t = 0$ months, black circles; $t = 1$ month, dark grey circles, $t = 13$ months, grey circles; $t = 26$ months, white circles). The increase in methane coincided with decreasing redox potentials (b; $t = 0$ months, black circles). After 13 months, redox potentials (grey circles) declined below $E_7 < 330\text{--}350$ mV indicating anoxic conditions (cf. Laanbroek, 1990) comparable with *Sphagnum* lawns. Error bars indicate SE, $n = 4$. Compare also with methane stocks in Fig. 2 and redox potential in Fig. 4.

Sphagnum vegetation, oxygen was absent when measured by mini-electrodes.

Clipping of cushion plants caused a significant change in soil processes. Within 1 month after clipping, $3 \mu\text{mol l}^{-1}$ (SE = 1.4, $n = 4$) methane accumulated in the uppermost rooting zone (Fig. 5a). After 13 months, the pore water concentration increased from zero to $c. 78 \mu\text{mol CH}_4 \text{ l}^{-1}$ (SE = 49) at 60 cm and $102 \mu\text{mol CH}_4 \text{ l}^{-1}$ (SE = 39) at 150 cm. This increase in methane concentration was accompanied by a drastic decrease in the redox potential to < 300 mV, suggesting a depletion of oxygen within a year

(Fig. 5b). After 26 months, mean methane emissions ($10 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$, $n = 6$) exceeded those of *Sphagnum* lawns (Fig. 1) and methane accumulated in the pore water to a concentration of $706 \mu\text{mol CH}_4 \text{ l}^{-1}$ at 60 cm (SE = 136, $n = 4$).

Methane oxidation and production

The activity of methanotrophs was found in the entire rooting zone of cushion plants. Mean activities ranged from 10 to $86 \mu\text{mol l}^{-1} \text{ d}^{-1}$ and 0.2 to $1.2 \mu\text{mol g}^{-1} \text{ DW d}^{-1}$, respectively (Fig. 6). The highest oxidation rates were found in the lower part of the rooting zone where oxygen was in the vicinity of methane-containing substrates (Figs 3, 4). The activity of methanotrophs ($10\text{--}15 \mu\text{mol l}^{-1} \text{ d}^{-1}$) was also found above the methane–oxygen interface. In the rooting zone, methanotrophs are methane limited (Fig. 3a), whereas, below the roots, methane consumption became oxygen limited (Fig. 4). Oxidation rates in the field may be 62% lower, assuming an average soil temperature of 8°C and a Q10 of 2, found for methanotrophs in the control bog by Kip *et al.* (2010).

Mean potential methane production in the upper 120 cm ranged from 1 to $20 \mu\text{mol l}^{-1} \text{ d}^{-1}$ and 0.04 to $0.36 \mu\text{mol g}^{-1} \text{ DW d}^{-1}$, respectively (Fig. 6). Based on volume, the highest production was found in the most recently accumulated parts: dense peat that was little decomposed and had the highest nutrient content. Because *Sphagnum* peat has a three to five times lower density (some $20\text{--}30 \text{ g l}^{-1}$), *Sphagnum* sites had a lower methane production potential per surface area or volume compared with cushion plants (density of $50\text{--}120 \text{ g l}^{-1}$). In aerobic incu-

bations, methane production was below the detection limit. Thus, the actual methane production is assumed to be negligible in the rooting zone of cushion plants because of aerobic conditions (Fig. 4).

Samples taken from the rooting zone of cushion plants showed a time-lagged increase in production, exceeding the volume-based rates of *Sphagnum* sites (Fig. 6). The time-lagged increase indicated that the community of methanogenic bacteria had adjusted to the anoxic conditions of the incubations. As the substrate from the rooting zone of cushion plants had been subjected to oxygen release, a low presence and activity of methanogens can be anticipated. Edwards *et al.* (1998) found that substrates from aerobic environments or exposed to oxygen after sampling exhibited hampered activity of methanogens.

Discussion

In this study, methane dynamics revealed a tight but inverse link to the presence of vascular plant roots. We found evidence that the specific conditions in cushion bogs lead to high oxygenation of the wetland soil well beyond the rhizosphere ($> 150 \text{ cm}$), thus limiting methane production and methane release via plants. Crucial for extensive oxygenation are the nutrient-poor conditions of these sites, limiting soil oxygen demand, combined with high densities of very long and aerenchymatous roots. These data are the first on methane emissions and stocks in temperate bogs in the Southern Hemisphere and Patagonia.

Cushion plants are a significant part of wetland and mountainous vegetation in the Southern Hemisphere (Gibson & Kirkpatrick, 1985; Blanco & de la Balze, 2004; Squeo *et al.*, 2006). Parts of these bogs consist of *Sphagnum* vegetation, and methane emissions from these sites and from a pure *Sphagnum* bog were low (Fig. 1). Wet lawns of *Sphagnum* ssp. emitted $1\text{--}14 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$, which is in the lower range reported for *Sphagnum*-dominated vegetation (reviewed in Saarnio *et al.*, 2009). The observed slow carbon and methane turnover can be explained by summer temperatures below 10°C (Daulat & Clymo, 1998; Segers, 1998), very low nutrient availability (Juottonen *et al.*, 2005; Schmidt *et al.*, 2010) and low pH (Segers, 1998).

The larger part of the cushion bogs consists of cushion plants, which are characterized by high densities of long aerenchymatous roots. These roots may function as a conduit for methane release. However, in the rooting zone of cushion plants, no methane was present, and only around this zone did methane levels increase steeply with (both horizontal and vertical) gradients of $200\text{--}300 \mu\text{mol l}^{-1} \text{ CH}_4 \text{ m}^{-1}$ (Figs 2, 3). Therefore, although a large methane stock was present at the ecosystem scale, methane emissions at cushion plant sites were low, approximating zero (Fig. 1). The main reason was the oxygenation of the rooting zone by oxygen loss from the roots (Fig. 4). Root-derived oxygen

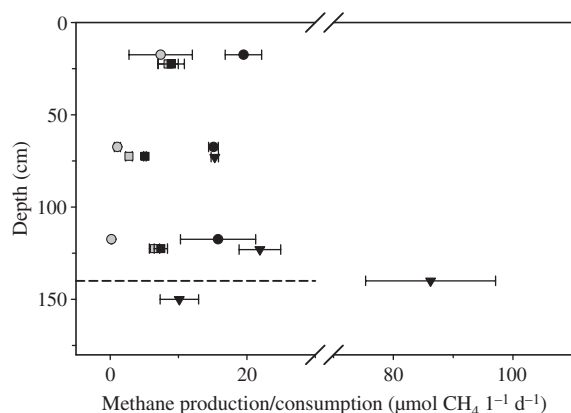


Fig. 6 Depth profile of methane (CH_4) oxidation rates that exceeded the rates of potential methane production at 22°C . After 2 wk, methane production of cushion plant peat (grey circles) was lower than that of *Sphagnum* peat (grey squares). By contrast, after 7 wk, cushion plants (black circles) revealed much higher potential methane production than *Sphagnum* peat (black squares). Methane oxidation rates (triangles) at 22°C were substantially higher at the lower boundary of the rooting zone (dashed line) of cushion plant site 1 (CP1). Error bars indicate SD, $n = 4$.

suppressed methane production and increased methane oxidation, thus diminishing methane stocks in the rooting zone (Fig. 6). By contrast, studies on root–methane interactions revealed that roots remained in contact with methane. This resulted in methane emission rates exceeding those common for *Sphagnum* vegetation (Popp *et al.*, 2000; Ding *et al.*, 2004; Strack *et al.*, 2006). Our study suggests that densely growing cushion plants have a higher potential to oxidize soil and methane than do common wetland species, for example *Phragmites* ssp. (van der Nat & Middelburg, 1998), *Oryza* ssp. (Frenzel, 2000), *Carex* ssp. (Popp *et al.*, 2000; Ding *et al.*, 2004) and *Sphagnum* ssp. (Larmola *et al.*, 2010). As a consequence of incomplete oxygenation, the methane production in anoxic parts of the soil becomes fuelled by easily decomposing root exudates and litter (Joabsson & Christensen, 2001; Juottonen *et al.*, 2005). This ‘fuelling-effect’ of vascular plants was tested in this study by long-term clipping of cushion plants. After cutting off the oxygen supply to the roots, the redox potential dropped well below 330 mV, indicating anoxic conditions in the rooting zone (Fig. 5). Consequently, a substantial methane stock built up within a few months in the upper 150 cm fuelled by decomposing roots. After 2 yr without oxygen supply, methane stocks exceeded those of *Sphagnum* vegetation (Figs 1, 6). Part of the built-up methane stock may have resulted from decaying roots, especially at the beginning of the experiment.

By contrast, living cushion plants can thoroughly oxygenate the organic peat soil through oxygen leakage from hundreds of root tips per litre of soil. Highly decomposed cushion bog peats are likely to consume little oxygen because of low soil temperatures (4–10°C) (Haraguchi, 1995; Chapman & Thurlow, 1998; Allen *et al.*, 2002) and carbon densities (50 g C l⁻¹). The recalcitrant nature of highly decomposed peat (Chapman & Thurlow, 1998) and the low nutrient availability, such as total P < 0.02% (Reddy *et al.*, 1999), further reduce oxygen consumption. The aerobic state of the soil prevailed in the upper 120 cm despite a decrease in root density with depth (Figs 3, 4). At root densities as low as two to five tips per litre, found in the bottom 10 cm of the rooting zone, an oxic state is unlikely to be maintained far beyond the root surface. However, this zone of low root density separates the bulk root surface from methane. We suggest that, in the upper profile, oxygen release rates exceed consumption. The surplus oxygen is transported by infiltrating rain water down the profile, where deeper root layers thus receive additional oxygen, next to the *in situ* oxygen leakage. Lateral groundwater flow can convey fairly oxidizing conditions beyond the rooting zone. This is indicated by lower methane stocks at the ecosystem level, as suggested by low methane concentration in the upper 200 cm below *Sphagnum* patches (Fig. 2) and pools (data not shown) in the cushion bog. This is further indicated by methanotrophic activity, which is maximal in the bottom 10 cm of the rooting

zone (Figs 3b, 6). Methanotrophic activity is highest where upward diffusion of methane meets available oxygen (Watson *et al.*, 1997; Edwards *et al.*, 1998). Despite the low root density, sufficient oxygen is present in the bottom 10 cm of the rooting zone to maintain methane oxidation (Figs 4, 6), which results in a spatial separation of roots and methane. When soil is aerated by deep drainage, a similar separation of roots and methane results in low or zero emissions (Roulet *et al.*, 1993; Bubier, 1995; Couwenberg *et al.*, 2010). A thorough oxygenation of the rooting zone is essential for sufficient separation between roots and methane stock and, consequently, for complete cessation of methane emission. This has also been suggested by physical models of root–methane interactions (Watson *et al.*, 1997; Segers *et al.*, 2001). Such oxygenation of wetland soils by an extensive and deep root biomass requires sufficient nutrients (van Bodegom *et al.*, 2005; Koelbener *et al.*, 2010) that pristine bogs usually lack (van Breemen, 1995; Kleinebecker *et al.*, 2008). Higher nutrient levels also increase oxygen consumption because litter/peat formed under nutrient-rich conditions breaks down more rapidly than recalcitrant *Sphagnum* litter from pristine sites (Aerts *et al.*, 1999; Chapin *et al.*, 2003). Incomplete oxygenation because of high soil oxygen consumption then permits the co-existence of roots and methane, resulting in methane emissions.

However, cushion plants, such as *Astelia* ssp. and *Donatia* ssp., have specific traits which allow them to develop a dense root system whilst still out-competing *Sphagnum* at low nutrient levels. These traits are a dense apical growth, high root to shoot ratio, very porous roots, low intrinsic growth rate, their evergreen nature and their efficient nutrient recycling (Gibson, 1990; Schmidt *et al.*, 2010; C. Fritz, unpublished). As a result of the high nutrient use efficiency and a low biomass turnover, a dense root system can be maintained even in the very nutrient-poor Patagonian bogs (Kleinebecker *et al.*, 2008; Schmidt *et al.*, 2010). We show that cushion plants can form two to four times more biomass of fine roots (3590 g DW m⁻²) than other bog vegetation (Moore *et al.*, 2002). A similar growth strategy is known from isoetid species growing at the bottom of nutrient-poor, soft-water lakes. Isoetid species (such as *Littorella uniflora* and *Lobelia dortmanna*) exhibit a dense root system whilst growing slowly, and also oxidize entire mineral soil layers (Smolders *et al.*, 2002). The mutual interaction between plant (traits) and soil conditions warrants further investigation.

Conclusion

From our study, we conclude that, under specific circumstances, vascular plants are capable of oxidizing the bulk of soil methane that might otherwise be released via the root aerenchyma. We have highlighted an outstanding example of an inverse relation between root density and methane

release. The clear spatial separation of methane from cushion plant roots resulted from the low oxygen consumption that was exceeded by oxygen loss from the roots. The influence of vascular plants on methane cycling depends on traits such as the formation of a dense root biomass in spite of nutrient-deficient conditions. Rising temperatures and habitat losses of cushion plants are expected to stimulate future methane emissions from Patagonian bogs.

Acknowledgements

For their invaluable support during field campaigns, all authors are much indebted to Victoria Surrur, Hernán Dieguez, Pablo Huelin Rueda, Hermen Keizer, Ronny Gebser and many others who made field work successful in extreme weather. We are grateful for the valuable comments of three anonymous referees. Facilities offered by the staff of the Prefectura Naval Argentina were highly appreciated. Important to us was the refrigerator, working space and logistics offered by Lucas Varela of the La Posta Hostal family. We would like to thank Gerard Bögemann for offering his redox equipment and expertise in measuring the porosity of roots. Susanne Abel is acknowledged for sharing her experience in taking root samples from cushion bogs. Assistance in methane incubation and isolation of methanotrophs by Nardy Kip was highly appreciated. Collaboration with CONICET was conducted within the Convenio XXI.

References

- Aerts R, Verhoeven JTA, Whigham DF. 1999. Plant-mediated controls on nutrient cycling in temperate fens and bogs. *Ecology* **80**: 2170–2181.
- Allen WC, Hook PB, Biederman JA, Stein OR. 2002. Temperature and wetland plant species effects on wastewater treatment and root zone oxidation. *Journal of Environmental Quality* **31**: 1010–1016.
- Armstrong J, Armstrong W, Beckett PM. 1992. *Phragmites australis* – Venturi-induced and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. *New Phytologist* **120**: 197–207.
- Armstrong J, Jones RE, Armstrong W. 2006. Rhizome phyllosphere oxygenation in *Phragmites* and other species in relation to redox potential, convective gas flow, submergence and aeration pathways. *New Phytologist* **172**: 719–731.
- Armstrong W, Justin S, Beckett PM, Lythe S. 1991. Root adaptation to soil waterlogging. *Aquatic Botany* **39**: 57–73.
- Blanco DE, de la Balze VM eds. 2004. *Los Turbales de la Patagonia, bases para su inventario y la conservación de su biodiversidad*. Buenos Aires, Argentina: Wetlands Internacional.
- van Bodegom PM, de Kanter M, Bakker C, Aerts R. 2005. Radial oxygen loss, a plastic property of dune slack plant species. *Plant and Soil* **271**: 351–364.
- Bortoluzzi E, Epron D, Siegenthaler A, Gilbert D, Buttler A. 2006. Carbon balance of a European mountain bog at contrasting stages of regeneration. *New Phytologist* **172**: 708–718.
- Bouchard V, Frey SD, Gilbert JM, Reed SE. 2007. Effects of macrophyte functional group richness on emergent freshwater wetland functions. *Ecology* **88**: 2903–2914.
- van Breemen N. 1995. How *Sphagnum* bogs down other plants. *Trends in Ecology and Evolution* **10**: 270–275.
- Breeuwer A, Heijmans M, Robroek BJM, Berendse F. 2010. Field simulation of global change: transplanting northern bog mesocosms southward. *Ecosystems* **13**: 712–726.
- Bubier JL. 1995. The relationship of vegetation to methane emission and hydrochemical gradients in Northern peatlands. *Journal of Ecology* **83**: 403–420.
- Chanton JP, Glaser PH, Chasar LS, Burdige DJ, Hines ME, Siegel DI, Tremblay LB, Cooper WT. 2008. Radiocarbon evidence for the importance of surface vegetation on fermentation and methanogenesis in contrasting types of boreal peatlands. *Global Biogeochemical Cycles* **22**: 1–11.
- Chapin CT, Bridgham SD, Pastor J, Updegraff K. 2003. Nitrogen, phosphorus, and carbon mineralization in response to nutrient and lime additions in peatlands. *Soil Science* **168**: 409–420.
- Chapman SJ, Thurlow M. 1998. Peat respiration at low temperatures. *Soil Biology and Biochemistry* **30**: 1013–1021.
- Couwenberg J, Dommain R, Joosten H. 2010. Greenhouse gas fluxes from tropical peatlands in south-east Asia. *Global Change Biology* **16**: 1715–1732.
- Darwin C. 1839. *Narrative of the surveying voyages of His Majesty's ships Adventure and Beagle, between the years 1826 and 1836, describing their examination of the southern shores of South America, and the Beagle's circumnavigation of the globe*. London, UK: Henry Colburn.
- Daulat WE, Clymo RS. 1998. Effects of temperature and water table on the efflux of methane from peatland surface cores. *Atmospheric Environment* **32**: 3207–3218.
- Ding WX, Cai ZC, Tsuruta H. 2004. Summertime variation of methane oxidation in the rhizosphere of a *Carex* dominated freshwater marsh. *Atmospheric Environment* **38**: 4165–4173.
- Ding WX, Cai ZC, Tsuruta H. 2005. Plant species effects on methane emissions from freshwater marshes. *Atmospheric Environment* **39**: 3199–3207.
- Edwards C, Hales BA, Hall GH, McDonald IR, Murrell JC, Pickup R, Ritchie DA, Saunders JR, Simon BM, Upton M. 1998. Microbiological processes in the terrestrial carbon cycle: methane cycling in peat. *Atmospheric Environment* **32**: 3247–3255.
- Frenzel P. 2000. *Plant-associated methane oxidation in rice fields and wetlands. Advances in microbial ecology*, Vol 16. New York, NY, USA: Kluwer Academic/Plenum Publication, 85–114.
- Fritz C, Campbell DI, Schipper LA. 2008. Oscillating peat surface levels in a restiad peatland, New Zealand – magnitude and spatiotemporal variability. *Hydrological Processes* **22**: 3264–3274.
- Gebser R. 2008. *Ecological studies on the vegetation distribution of the Moat cushion peatland in Tierra del Fuego, Argentina*. MSc Thesis, University of Greifswald.
- Gibson N. 1990. The environments and primary production of cushion species at Mt Field and Mt Wellington, Tasmania. *Australian Journal of Botany* **38**: 229–243.
- Gibson N, Kirkpatrick JB. 1985. A comparison of the cushion plant-communities of New-Zealand and Tasmania. *New Zealand Journal of Botany* **23**: 549–566.
- Godoy R, Paulino L, Oyarzun C, Boeckx P. 2003. Atmospheric N deposition in central and southern Chile: an overview. *Gayana Botánica* **60**: 47–53.
- Grootjans AP, Iturraspe R, Lanting A, Fritz C, Joosten H. 2010. Ecohydrological features of some contrasting mires in Tierra del Fuego, Argentina. *Mires and Peat* **6**: 1–15.
- Grosse W, Jovy K, Tiebel H. 1996. Influence of plants on redox potential and methane production in water-saturated soil. *Hydrobiologia* **340**: 93–99.
- Haraguchi A. 1995. Seasonal-changes in oxygen-consumption rate and redox property of floating peat in a pond in central Japan. *Wetlands* **15**: 242–246.
- Hornibrook ERC, Bowes HL, Culbert A, Gallego-Sala AV. 2009. Methanotrophy potential versus methane supply by pore water diffusion in peatlands. *Biogeosciences* **6**: 1491–1504.

- Iturraspe R, Sottini R, Schröder R, Escobar J. 1989. Hidrología y variables del territorio de Tierra del Fuego – Información básica. *CADIC-CONICET, Contribución Científica* 7: 1–196.
- Joabsson A, Christensen TR. 2001. Methane emissions from wetlands and their relationship with vascular plants: an Arctic example. *Global Change Biology* 7: 919–932.
- Joabsson A, Christensen TR, Wallen B. 1999. Vascular plant controls on methane emissions from northern peatforming wetlands. *Trends in Ecology and Evolution* 14: 385–388.
- Johansson T, Malmer N, Crill PM, Friberg T, Akerman JH, Mastepanov M, Christensen TR. 2006. Decadal vegetation changes in a northern peatland, greenhouse gas fluxes and net radiative forcing. *Global Change Biology* 12: 2352–2369.
- Juottonen H, Galand PE, Tuittila ES, Laine J, Fritze H, Yrjala K. 2005. Methanogen communities and Bacteria along an ecophysiological gradient in a northern raised bog complex. *Environmental Microbiology* 7: 1547–1557.
- Kao-Kniffin J, Freyre DS, Balser TC. 2010. Methane dynamics across wetland plant species. *Aquatic Botany* 93: 107–113.
- King GM. 1994. Associations of methanotrophs with the roots and rhizomes of aquatic vegetation. *Applied and Environmental Microbiology* 60: 3220–3227.
- Kip N, van Winden JF, Pan Y, Bodrossy L, Reichart GJ, Smolders AJP, Jetten MSM, Damste JSS, Op den Camp HJM. 2010. Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. *Nature Geoscience* 3: 617–621.
- Kleinebecker T, Hölzel N, Vogel A. 2007. Gradients of continentality and moisture in South Patagonian ombrotrophic peatland vegetation. *Folia Geobotanica* 42: 363–382.
- Kleinebecker T, Hölzel N, Vogel A. 2008. South Patagonian ombrotrophic bog vegetation reflects biogeochemical gradients at the landscape level. *Journal of Vegetation Science* 19: 151–160.
- Koelbener A, Ström L, Edwards PJ, Venterink HO. 2010. Plant species from mesotrophic wetlands cause relatively high methane emissions from peat soil. *Plant and Soil* 326: 147–158.
- Kutzbach L, Wagner D, Pfeiffer EM. 2004. Effect of microrelief and vegetation on methane emission from wet polygonal tundra, Lena Delta, Northern Siberia. *Biogeochemistry* 69: 341–362.
- Laanbroek HJ. 1990. Bacterial cycling of minerals that affect plant-growth in waterlogged soils: a review. *Aquatic Botany* 38: 109–125.
- Laanbroek HJ. 2009. Methane emission from natural wetlands: interplay between emergent macrophytes and soil microbial processes. A mini-review. *Annals of Botany* 105: 141–153.
- Larmola T, Tuittila ES, Tirola M, Nykanen H, Martikainen PJ, Yrjala K, Tuomivirta T, Fritze H. 2010. The role of *Sphagnum* mosses in the methane cycling of a boreal mire. *Ecology* 91: 2356–2365.
- Lloyd D, Thomas KL, Benstead J, Davies KL, Lloyd SH, Arah JRM, Stephen KD. 1998. Methanogenesis and CO₂ exchange in an ombrotrophic peat bog. *Atmospheric Environment* 32: 3229–3238.
- Moore TR, Bubier JL, Frothingham SE, Lafleur PM, Roulet NT. 2002. Plant biomass and production and CO₂ exchange in an ombrotrophic bog. *Journal of Ecology* 90: 25–36.
- van der Nat F, Middelburg JJ. 1998. Effects of two common macrophytes on methane dynamics in freshwater sediments. *Biogeochemistry* 43: 79–104.
- Popp TJ, Chanton JP, Whiting GJ, Grant N. 2000. Evaluation of methane oxidation in the rhizosphere of a *Carex* dominated fen in north central Alberta, Canada. *Biogeochemistry* 51: 259–281.
- Raghoebarsing AA, Smolders AJP, Schmid MC, Rijpstra WIC, Wolters-Arts M, Derksen J, Jetten MSM, Schouten S, Damste JSS, Lamers LPM *et al.* 2005. Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. *Nature* 436: 1153–1156.
- Reddy KR, White JR, Wright A, Chua T. 1999. Influence of phosphorus loading on microbial processes in the soil and water column of wetlands. In: Reddy KR, O'Connor GA, Schelske CL, eds. *Phosphorus biogeochemistry in subtropical ecosystems*. Boca Raton, FL, USA: CRC Press-Taylor & Francis Group, 249–273.
- Roig CE, Collado L. 2004. Ventana N° 7 – Moat. In: Blanco DE, de la Balze VM, eds. *Los Turbales de la Patagonia, bases para su inventario y la conservación de su biodiversidad*. Buenos Aires, Argentina: Wetlands International, 66–71.
- Roulet NT, Ash R, Quinton W, Moore T. 1993. Methane flux from drained northern peatlands – effect of a persistent water-table lowering on flux. *Global Biogeochemical Cycles* 7: 749–769.
- Saarnio S, Winiwarter W, Leitaio J. 2009. Methane release from wetlands and watercourses in Europe. *Atmospheric Environment* 43: 1421–1429.
- Schmidt SR, Kleinebecker T, Vogel A, Hölzel N. 2010. Interspecific and geographical differences of plant tissue nutrient concentrations along an environmental gradient in Southern Patagonia, Chile. *Aquatic Botany* 92: 149–156.
- Segers R. 1998. Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry* 41: 23–51.
- Segers R, Rappoldt C, Leffelaar PA. 2001. Modeling methane fluxes in wetlands with gas-transporting plants 2. Soil layer scale. *Journal of Geophysical Research-Atmospheres* 106(D4): 3529–3540.
- Smolders AJP, Lucassen E, Roelofs JGM. 2002. The isoelectric environment: biogeochemistry and threats. *Aquatic Botany* 73: 325–350.
- Sorrell BK, Downes MT, Stanger CL. 2002. Methanotrophic bacteria and their activity on submerged aquatic macrophytes. *Aquatic Botany* 72: 107–119.
- Sorrell BK, Tanner CC, Sukias JPS, Roberts J. 2001. How does your wetland grow?: growth and morphological responses of emergent wetland plants to flooded soils and water depth. In: IEES, eds. *International ecological engineering conference*. Lincoln, New Zealand: IEES, 291–295.
- Squeo FA, Warner BG, Aravena R, Espinoza D. 2006. Bofedales: high altitude peatlands of the central Andes. *Revista Chilena de Historia Natural* 79: 245–255.
- Strack M, Waller MF, Waddington JM. 2006. Sedge succession and peatland methane dynamics: a potential feedback to climate change. *Ecosystems* 9: 278–287.
- Ström L, Ekberg A, Mastepanov M, Christensen TR. 2003. The effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. *Global Change Biology* 9: 1185–1192.
- Ustin SL, Gamon JA. 2010. Remote sensing of plant functional types. *New Phytologist* 186: 795–816.
- Visser EJW, Bögemann GM. 2003. Measurement of porosity in very small samples of plant tissue. *Plant and Soil* 253: 81–90.
- Visser EJW, Bögemann GM, van de Steeg HM, Pierik R, Blom C. 2000. Flooding tolerance of *Carex* species in relation to field distribution and aerenchyma formation. *New Phytologist* 148: 93–103.
- Waddington JM, Roulet NT, Swanson RV. 1996. Water table control of CH₄ emission enhancement by vascular plants in boreal peatlands. *Journal of Geophysical Research-Atmospheres* 101(D17): 22775–22785.
- Watson A, Stephen KD, Nedwell DB, Arah JRM. 1997. Oxidation of methane in peat: kinetics of CH₄ and O₂ removal and the role of plant roots. *Soil Biology and Biochemistry* 29: 1257–1267.
- Whalen SC. 2005. Biogeochemistry of methane exchange between natural wetlands and the atmosphere. *Environmental Engineering Science* 22: 73–94.
- Whiting GJ, Chanton JP. 1993. Primary production control of methane emission from wetlands. *Nature* 364: 794–795.
- Woodin SJ, van der Wal R, Sommerkorn M, Gornall JL. 2009. Differential allocation of carbon in mosses and grasses governs ecosystem sequestration: a ¹³C tracer study in the high Arctic. *New Phytologist* 184: 944–949.